The Effects of Several Forms of Transplantable Rat Leukemias on the Carbohydrate Metabolism of the Host

HEATHER DUNCAN SEAY and HARRIS ROSENKRANTZ

Department of Biology, Clark University, and the Mason Research Institute, Worcester, Massachusetts

SUMMARY

The dependence of transplantable neoplasms on the host's carbohydrate metabolism was explored in rat lymphocytic leukemia, WR-6, myelogenous leukemia, LW-12, and a monocytic type of leukemia, R-3149. Implants were made with tissue slices or leukemic blood. Liver glycogen, plasma glucose, and lactate were followed at several postimplantation intervals. The WR-6 type was studied in more detail in relation to diet, the course of the malignancy, and the presence or absence of a tumor mass. It was found that the WR-6 evoked a severe hypoglycemia with depletion of glycogen stores. Rats bearing the leukemia LW-12 tended to resist a change in blood glucose levels while the short survival time of the R-3149 prevented emergence of carbohydrate derangement. Elevated blood lactate levels pointed to conversion by tumor tissue of the host's glucose in a wasteful process of energy production at the expense of the host's ability to survive.

Since the characterization of 3 types of leukemia was taking place at the Mason Research Institute, it was of considerable interest to compare their influence on the host's carbohydrate metabolism. To this end blood glucose, liver glycogen, and in some instances blood lactate were determined. The parameters for one leukemia were followed over a time period, and some measurements in relation to the type of inoculum and site of implantation were made.

MATERIALS AND METHODS

Tumors were routinely transplanted so that tissue was available at a similar growth phase. Sterile precautions were observed, and grossly necrotic or fibrous areas were removed before implantation.

The lymphocytic leukemia designated WR-6 was transferred into 50- to 68-day-old male rats of the inbred Wistar-Furth/Schmidt (WFu/SCF) strain by s.c. inoculation of a 0.2-ml saline tumor mince with a No. 13 trocar. The WR-6 tumor system has been characterized as a lymphoblastic leukemia inducing poor body growth, a tumor mass at the site of inoculation within 5—7 days, elevation of leukocytes in excess of 200,000 cells/cu mm, and a survival time of 14—18 days postimplantation (DPI). The original tumor arose spontaneously in Dr. Jacob Furth's inbred strain of Wistar rats (11).

The stability and availability of the WR-6 tumor permitted a time study during which the biochemical parameters could be estimated in rats sacrificed 3, 6, 9, and 12 DPI. In another direction an attempt was made to induce a peripheral leukemic picture without the complication...
of a tumor mass. A donor with a total WBC of 275,000 cells/cu mm was selected at 14 DPI, and blood was taken by cardiac puncture. One-tenth ml of the blood was injected i.p. into each of five 64-day-old male WFu/SCF rats (9). In a 2nd study 0.1 ml of heparinized leukemic blood from a similar donor was injected into the hearts of 8 recipients. All animals were sacrificed 9 DPI.

In order to gain some knowledge of the influence of diet on the development of the WR-6, 40 rats were implanted with tumor tissue and 2 groups were arranged for paired feeding. Ten animals in each group received weighed amounts of powdered laboratory chow, and after determination of food consumption, the other 10 animals in each group were given half the amount of food consumed.

Twenty rats were sacrificed at 9 DPI and 20 at 14 DPI.

A myelogenous leukemia, LW-12, which also arose in Dr. Furth’s inbred rats was the 2nd tumor system studied. Transplantation was carried out by s.c. implantation of 2 slices of tumor, 5 x 10 x 0.1 mm, through a dorsal incision. The donor animals were 29 DPI and the recipients 50- to 68-day-old male, inbred WFu/SCF rats. Animals were sacrificed at 34 DPI.

Partial characterization of the LW-12 indicates a latent period of 18–21 days, after which tumor growth is rapid. Elevation of immature granulocytes occurs, and the survival time is between 30 and 40 days.  

The 3rd leukemia investigated was a monocytic type maintained in inbred Fischer 344 strain of rats (F/CHA) and designated R-3149. The tumor was received from Dr. Wilhelmina Dunning in 1963 and is transplanted as a splenic mince (4). Partial characterization suggests a survival time of 7–11 days with WBC’s approaching 60,000 cells/cu mm. In contrast to the WR-6 and LW-12 tumor systems, which yield relatively large tumor masses at term, the R-3149 tumor remains small. The course of the disease involves rapid infiltration and metastasis rather than pronounced tumor growth.  

For the present study 34- to 43-day-old, inbred female F/CHA rats received one 2- to 3-cu-mm slice of R-3149 splenic tissue from a donor carrying the tumor mass for 7–9 days. Sacrifice was at 8 DPI.

At the appropriate time controls and animals with the various leukemias were anesthetized with ether, and a blood sample was taken by cardiac puncture. Coagulation was prevented by 5% disodium ethylenediaminetetraacetate in isotonic saline. The red blood cells were removed by centrifugation. The glucose oxidase method of Saifer and Gerstenfeld (13) was employed for the determination of plasma glucose; a commercial kit by Dade Reagents, Inc., was used. Plasma lactate was estimated by the method of Umbreit et al. (15).

After the sample of cardiac blood was taken, the animals were killed by cervical dislocation. The liver was resected, blotted, weighed, and digested in 10 ml of 30% potassium hydroxide. A modification of the method of Seifter et al. (14) was utilized for glycogen estimation. It was found that 0.2% anthrone in 73%, rather than 95%, sulfuric acid gave more intense color products. A comparison was made between the direct determination of glycogen in diluted digests and measurement on resolubilized ethanol precipitates. Known quantities of exogenous glycogen were added to 1 ml of 10-ml digests of total livers.

## RESULTS

The desire to apply a simple but specific procedure for glycogen determination made it obligatory to perform recovery experiments. Livers (7.23 ± 0.24 gm) from normal rats, each containing 216 ± 13 mg of glycogen, were used. One ml of a 10-ml digest received the exogenous glycogen, and values shown were corrected for endogenous glycogen levels. These results are presented in Table 1 and clearly establish that, over a wide range (13–200 μg) of exogenous glycogen additions to alkali solutions or liver digests, recovery values ranged from 92 to 108%.

Furthermore, it was found that glycogen purification through ethanol precipitation was not necessary for accurate measurement of glycogen. In 3 separate comparative studies involving a total of 38 analyses in duplicate on Wistar-Furth and Sprague-Dawley rat livers, the following means and standard deviations were obtained: direct analysis of 1:100, 1:50, or 1:10 dilutions of liver digests, 320 ± 10, 190 ± 18, and 227 ± 30 mg. The corresponding values for resolubilized glycogen precipitates from 1:10 dilutions were 290 ± 10, 180 ± 15, and 207 ± 27 mg. The difference between the 2 methods was between 5 and 10%. Only 10–20% of the glycogen could be precipitated by ethanol when liver digest dilutions of 1:50 or 1:100 were employed.

In Table 1 are also shown recovery values for glucose added to rat plasma, as well as the precision achieved with commercial standard preparations (Lab-Trol). Recoveries were between 96 and 108%. A few recoveries of exogenous lactate were carried out and were approximately 100%.

Animals at intermediate and terminal stages of tumor growth were investigated in 3 leukemic systems. Determinations of liver glycogen, plasma glucose, and lactate...
The relationship of the loss of liver glycogen and plasma glucose occurred. With the progression of the malignancy a startling 95% deprivation of glycogen takes place and is accompanied by a marked fall (67-72%) in glucose and a 179% rise in blood lactate.

In the instance of the LW-12 leukemia a modest decline in liver glycogen was evoked between 18-35 DPI. An inconsistent change in blood glucose occurred, while lactate increased by 93% at 35 DPI.

The 3rd leukemia, R3149, was characterized by steady decrements in liver glycogen with time of tumor growth. No change was apparent for circulating glucose, but blood lactate began to go up (42%) by 10 DPI.

For general information, it is worthwhile to insert control values of glycogen and glucose obtained on other rat strains. In 200-gm normal SD/SPD rats liver glycogen was 216 ± 3 and plasma glucose, 160 ± 6; for 120-gm SD/CHA the values were 175 ± 12 and 132 ± 21, respectively. These findings may be compared with those in Table 2 for normal 122- to 139-gm WFu/SCF animals, where liver glycogen was between 157 and 227 mg/gm wet weight, whereas older ones (148 gm) had values near 171 and 169, respectively. Some variation in liver glycogen content for all strains may be attributed to individual eating habits.

A more detailed investigation of glycogen and glucose levels in terms of the time of tumor development was made. Since the WR-6 system showed the most profound changes, it was selected for additional study. Results on the influence of time for the WR-6 are given in Table 3. A decline in both biochemical parameters became evident on Day 6 and continued to drastically reduced levels on Day 14.

Since the WR-6 lymphocytic leukemia was associated with a solid tumor, it was of interest to determine whether a similar biochemical picture could be elicited in the absence of a tumor mass. This was achieved by i.p. (ascites) or intracardial (systemic) injection of leukemic blood, and the results are outlined in Table 4. It can be seen that the ascites form of WR-6 yielded a nearly iden-

### Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of rats</th>
<th>DPI</th>
<th>Age (days)</th>
<th>Body wt (gm)</th>
<th>Liver wet wt (gm)</th>
<th>Glycogen in total liver (mg)</th>
<th>Plasma glucose (mg/100 ml)</th>
<th>Plasma lactate (mg/ml)</th>
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<td>Control</td>
<td>30</td>
<td>52 ± 3</td>
<td>122 ± 12</td>
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<td>179 ± 49</td>
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<td>33 ± 12</td>
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<td>30</td>
<td>9</td>
<td>115 ± 10</td>
<td>5.61 ± 0.57</td>
<td>107 ± 32</td>
<td>102 ± 7</td>
<td>34 ± 9</td>
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<tr>
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<td>15</td>
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<td>5.87 ± 0.44</td>
<td>227 ± 38</td>
<td>147 ± 12</td>
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<tr>
<td>WR-6</td>
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<td>67 ± 1</td>
<td>127 ± 10</td>
<td>10.09 ± 0.82</td>
<td>13 ± 2</td>
<td>48 ± 15</td>
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<tr>
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<td>57 ± 3</td>
<td>133 ± 8</td>
<td>5.61 ± 0.61</td>
<td>157 ± 59</td>
<td>117 ± 10</td>
<td>33 ± 10</td>
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<td>121 ± 12</td>
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<td>33 ± 9</td>
<td>92 ± 12</td>
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<tr>
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<td>123 ± 10</td>
<td>32 ± 9</td>
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<tr>
<td>LW-12</td>
<td>30</td>
<td>18</td>
<td>53 ± 3</td>
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<td>7.00 ± 1.10</td>
<td>101 ± 45</td>
<td>155 ± 14</td>
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<tr>
<td>Control</td>
<td>15</td>
<td>53 ± 3</td>
<td>139 ± 13</td>
<td>5.87 ± 0.44</td>
<td>227 ± 38</td>
<td>147 ± 12</td>
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<tr>
<td>LW-12</td>
<td>4</td>
<td>34</td>
<td>101 ± 0</td>
<td>216 ± 21</td>
<td>10.18 ± 0.63</td>
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<td>144 ± 4</td>
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<td>97 ± 23</td>
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<td>80 ± 7</td>
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<td>7</td>
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<td>3.43 ± 0.45</td>
<td>117 ± 32</td>
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<tr>
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<td>63 ± 3</td>
<td>148 ± 16</td>
<td>6.05 ± 0.51</td>
<td>171 ± 24</td>
<td>169 ± 18</td>
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<td>R-3149</td>
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<td>8</td>
<td>48 ± 3</td>
<td>92 ± 6</td>
<td>3.82 ± 0.20</td>
<td>123 ± 13</td>
<td>161 ± 15</td>
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<tr>
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<td>93 ± 9</td>
<td>3.92 ± 0.50</td>
<td>111 ± 25</td>
<td>95 ± 11</td>
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</tr>
<tr>
<td>R-3149</td>
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<td>10</td>
<td>46 ± 3</td>
<td>92 ± 8</td>
<td>4.19 ± 0.45</td>
<td>30 ± 18</td>
<td>98 ± 11</td>
<td>41 ± 17</td>
</tr>
</tbody>
</table>

* WFu/SCF rats were used for the WR-6 and LW-12 leukemias: F/CHA rats were used for R-3149.

### Table 3

The relationship of the loss of liver glycogen and plasma glucose to the time of onset of WR-6 leukemia

<table>
<thead>
<tr>
<th>DPI</th>
<th>Age (days)</th>
<th>Body wt (gm)</th>
<th>Liver wet wt (gm)</th>
<th>Glycogen in total liver (mg)</th>
<th>Plasma glucose (mg/100 ml)</th>
<th>Plasma lactate (mg/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>53 ± 3</td>
<td>139 ± 12</td>
<td>5.87 ± 0.48</td>
<td>227 ± 31</td>
<td>147 ± 12</td>
<td>33 ± 12</td>
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<tr>
<td>3</td>
<td>69 ± 0</td>
<td>185 ± 13</td>
<td>7.38 ± 0.31</td>
<td>195 ± 13</td>
<td>139 ± 3</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>6</td>
<td>72 ± 0</td>
<td>197 ± 12</td>
<td>7.10 ± 0.75</td>
<td>208 ± 54</td>
<td>146 ± 3</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>9</td>
<td>75 ± 0</td>
<td>183 ± 6</td>
<td>6.90 ± 0.18</td>
<td>103 ± 15</td>
<td>114 ± 6</td>
<td>33 ± 10</td>
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<tr>
<td>12</td>
<td>78 ± 0</td>
<td>164 ± 9</td>
<td>7.52 ± 0.48</td>
<td>41 ± 18</td>
<td>66 ± 6</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>13</td>
<td>77 ± 1</td>
<td>178 ± 9</td>
<td>9.52 ± 0.63</td>
<td>14 ± 2</td>
<td>57 ± 15</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>14</td>
<td>77 ± 0</td>
<td>184 ± 8</td>
<td>11.12 ± 0.47</td>
<td>11 ± 1</td>
<td>32 ± 6</td>
<td>33 ± 10</td>
</tr>
</tbody>
</table>

* For 0 and 13 days postimplantation, the mean value represents 15 and 9 Wistar-Furth male rats, respectively, while 5 animals were used for all other measurements.

** Mean ±S.D.
The paired-feeding study revealed the expected stress of anorexia on liver glycogen deposits and on tumor and body weights. However, plasma glucose levels were similar in all groups. The hypoglycemia and elevation of lactate in the blood was less pronounced in the partially starved animals at 14 DPI because tumor size was inhibited.

The elevation in plasma lactate in the 3 types of leukemia pointed to the wasteful metabolism of host glucose by the tumor tissue. Anaerobic glycolysis appears to be the preferred route of glucose metabolism, and it has been observed that WR-6 tumor slices utilize oxygen at a slower rate than normal tissues. This neoplasm also had poor glycogen depots and significant quantities of tissue lactate.

Busch et al. (3) have suggested that differences in lactate production by tumors and other tissues in vivo are not due to differences in mechanisms or rates of glycolysis but can be attributed to the inability of the tumor to reoxidize lactate under the environmental conditions of tumor growth.

The lack of a solid tumor mass in the ascites form of the WR-6 leukemia was consistent with a decrease in liver glycogen and plasma glucose. It was reasonable to assume that the ascites cells were capable of obtaining circulating glucose from the rich supply of capillaries in the lining membranes and mesenteric tissues found in the abdominal cavity. Evidence for such a free exchange has been discussed by Bloch-Frankenthal and Weinhouse (2) and Kemp and Mendel (10).

The results of investigations by Freedland and Waisman (5, 6) were of direct importance to the leukemias discussed above. These workers explored chemically induced leukemias that had no tumor mass, contained a minimum of ascites cells (J strain) or occasional ascites (strain 302), and developed through general infiltration. In addition to the marked elevation of circulating lymphocytes, liver glycogen and blood glucose fell drastically. In their rats it was found that 24 hr prior to death the liver glycogen decreased from a control of 4.4 to nearly 0% in both the 302 and J strains. Blood sugar values, determined by the less specific Somogyi’s method, dropped

- 4 H. Rosenkrantz and R. Sprague, unpublished data.
- 5 H. Rosenkrantz and J. H. Ellis, Jr., unpublished data.
from a control level of 102 to 24 for the 302 strain and to 37 mg % for the J strain.

Freedland and Waisman (6) also reported a marked elevation of blood lactate in their leukemic rats. Moreover, increased activities of glucose-6-phosphatase and fructose-1,6-diphosphatase were found. The changes in the various biochemical parameters occurred before signs of anorexia. The levels of liver glycogen and blood glucose varied in time with the inverse alteration of blood lactate, glycolytic enzymes, and white blood cells. The latter were excluded as a source of the increased glucose-6-phosphatase and fructose-1,6-diphosphatase activity found. These authors also noted that the rats bearing either strain of leukemia showed a marked drop in hemoglobin paralleling that of glucose just prior to the increase found in lactic acid. It was suggested that the decrease in hemoglobin with its decrease in available oxygen could inhibit the normal rate of oxidation and result in increased anaerobic glycolysis for the production of energy. This was supported by the high level of blood lactate.

The over-all picture that develops suggests that the leukemia having the most rapid rate of growth (WR-6 with tumor mass or ascites form) readily drains glucose from the host’s circulation. In turn liver glycogen depots are emptied in order to maintain the strict requirement of blood glucose levels. Neither dietary carbohydrates (in part limited by anorexia) nor gluconeogenesis can keep up with the tumor’s demands for glucose. The tumor cells primarily oxidize glucose to lactate for synthetic fragments and energy production, returning the lactate to the host’s circulation.

In the case of the R-3149, the relatively short survival time and small tumor masses achieved suggest extensive infiltration with little time to cause serious carbohydrate derangement in the host. In instances in which carbohydrate metabolism is challenged and insulted, hypoglycemia must be contributory in the death of the host.

ACKNOWLEDGMENT

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